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The Pulmonary Extracellular Lining

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The extracellular lining of the lungs is reviewed. The pulmonary extracellular lining is a complex mixture of phospholipids, proteins and carbohydrates which is absolutely essential for the maintenance of normal pulmonary functions such as gas exchange. Without the lining the lungs would collapse. Alterations in the pulmonary extracellular lining may underlie some disease conditions induced by toxic agents, especially those which interfere with the formation of pulmonary surfactant. The extracellular lining could be used to detect and monitor damage and disease caused by agents toxic to the lungs. The lining contains many hydrolytic enzymes which may act to detoxify certain toxic agents such as those which contain ester groups. The pulmonary extracellular lining could play a significant role mediating the toxic action of inhaled agents as well as the removal of those agents from the lungs.

Introduction

The extracellular lining (EL) of the lungs covers the epithelium of the terminal bronchioles and alveoli. The EL is a complex mixture of biomolecules, primarily lipids and proteins. As the EL is at least partly derived from the lung epithelium, and these cells are often the primary target of toxic agents, then qualitative and/or quantitative differences in components of the EL may be useful as indicators of changes in epithelial cell numbers or functions as part of the response to toxicants. In addition, changes in the EL may also give an insight into the mode of action of toxic agents on the lung, which is often unknown at the molecular level.

The modern era of research into the EL began with von Neergaard in 1929 (*1*). He demonstrated that surface tension forces played an important role in normal lung mechanics, and he postulated that a lowered surface tension was present at the tissue–air interface. However, no further significant studies were made until the 1950s. In 1953 (*2*), Low showed for the first time that the alveolar epithelium consisted of a continuous layer of cells, and in 1954 Macklin (*3*) observed that this epithelium was covered with a thin film of fluid. Then, pioneering studies by Pattle (*4,5*) proved that a substance, now usually called (pulmonary) surfactant, was present on the distal lung surfaces, that the surfactant was capable of lowering surface tension to very low levels, and that the surfactant was essentially a specific lung secretion. Other investigations, primarily by Clements and co-workers (*6–9*), proved that lowered surface tension was essential for lung function; if the surface tension was too high then the lung alveoli tended to collapse at low volumes, i.e., at end-expiration. Surfactant also lines the terminal bronchioles, preventing them from collapse at low volumes (*10*). The vital importance of surfactant was evidenced by the finding that surfactant was functionally deficient in newborn babies suffering from respiratory distress syndrome (*11–13*), a disease primarily characterized by diffuse atelectasis (lung collapse) in the affected infants. During the early 1960s, it was realized that surfactant was composed mainly of phospholipid (*14,15*), the major phospholipid being dipalmitoyl–phosphatidylcholine (*16*). Macklin in the early 1950s (*3,17*) suggested the Type II alveolar cell as the source of surfactant, but it was not until the 1970s that this was established with certainty. It is now known that surfactant is stored in Type II cell organelles called lamellar bodies, which are subsequently secreted into the aqueous subphase of the EL (*18–22*). Once in the subphase the lamellar bodies disrupt, perhaps into a structure called tubular myelin (*23,24*), and ultimately a thin monomolecular layer of surfactant is formed at the air–liquid interface. However, the site(s) of degradation of surfactant is still unknown. The most likely site was considered to be the alveolar macrophage (*25*), but this suggestion has not been supported by experimental evidence (*26,27*). It now seems that most surfactant is absorbed by cells(s) of the lung parenchyma (*26,27*). During the last 10 years, it has become apparent that the EL contains many nonsurfactant-associated proteins, most of which are plasma-derived (*28–30*), and also some enzymes (*31*). In addition to the work described here, many studies have been performed on aspects of surfactant metabolism and control but these studies are outside the scope of this review.

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Histology of the Alveolus

The alveolar epithelium mainly consists of two types of cells (*32,33*) (Fig. 1). The type I cell (membranous pneumonocyte) is a squamous cell. In the central region of the cell is the nucleus and a small number of organelles, e.g., lysosomes, mitochondria. From this central region arise extensive outpushings of the cell which cover over 90% of the alveolar surface. The Type I cell is considered to be metabolically inactive (*32,33*). The Type II cell (granular pneumonocyte) is metabolically highly active and is the source of the pulmonary surfactant. The cell is roughly cuboidal in shape, has microvilli on the apical surface and contains lamellar bodies (*32,33*). Although the two kinds of cell are present in about equal numbers in the normal alveoli, the vast surface area of the Type I cells mean that less than 10% of the gas-exchanging surface is represented by Type II cells (*32,33*). Overlying the surface of both cell types (but thicker over the Type II cells) is a tightly bound carbohydrate-rich glycocalyx, consisting mainly of glycosaminoglycans (*34–37*). The alveolar macrophage is also present in the alveoli, but as a free-moving cell on top of the epithelium. It has a defensive function in the lung, attempting to remove particles, bacteria, etc., that are deposited in the alveoli (*38,39*).

The EL overlies the epithelium (Fig. 1). In 1959, Chase (*40*) first reported the presence of an extracellular layer on the alveolar surface but technical problems with fixation of the layer prevented further progress until the work of Gil and Weibel (*41,42*) and others (*43, 44*). It was found that the EL had two components: (1) a hypo(epi) phase which contained lamellar bodies and tubular myelin and which was thought to be mainly aqueous in nature and (2) a surface film (*41,42*). The hypophase tended to be deeper in the alveolar "niches"

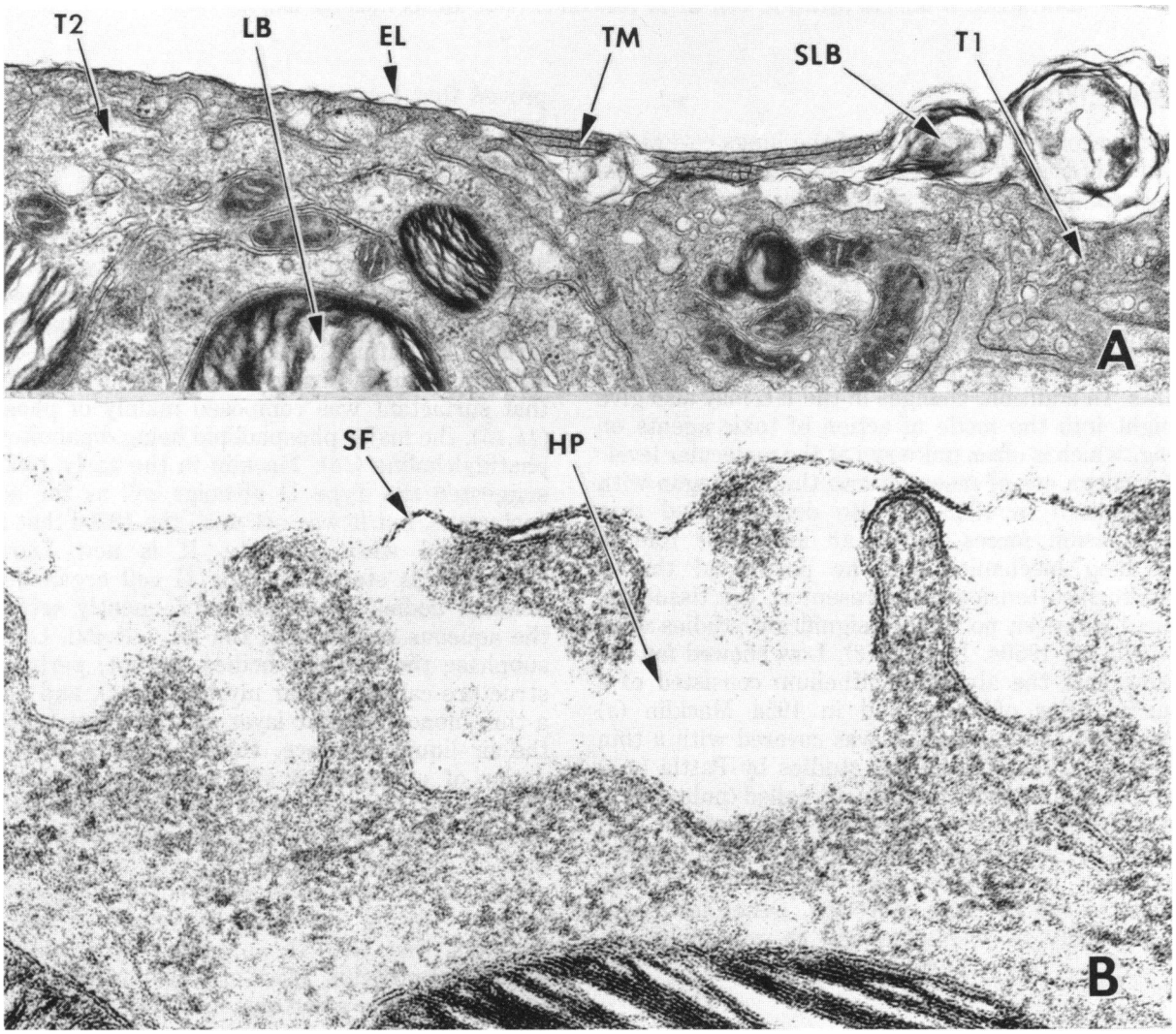

FIGURE 1. Extracellular lining of pulmonary alveoli of the rabbit. (*A*) Extracellular lining (EL) overlies a Type II (T2) and Type I (T1) cell. The Type II cell contains lamellar bodies (LB) that are secreted into the alveoli. Two secreted lamellar bodies (SLB) are shown adjacent to some tubular myelin (TM). ×25,000 .(*B*) The basic organization of the extracellular lining consists of the surface film (SF) and underlying hypophase (HP). ×108,000.

and also seemed to contain protein. The hypophase was overlaid by the surface film, a thin osmiophilic layer which presumably represented the surfactant monomolecular layer, consisting mainly, if not entirely, of phospholipid (Fig. 1). The EL of the terminal bronchioles also apparently possesses a monolayer of surfactant (*10, 45*). The hypophase in the bronchioles may contain a protein-rich secretion from the bronchiolar Clara cell (*46,47*); however, nothing is known about the nature of this secretion.

Lamellar Bodies

The lamellar bodies present in Type II cells were first suggested to be the intracellular storage form of surfactant by Macklin (*3*). Since that time, many morphological and biochemical studies have confirmed this supposition (*18–22,48–50*). Lamellar bodies seem to be modified lysosomes, since they contain acid hydrolases (*20,51–53*) and appear to originate from multivesicular bodies (*53,54*). A mature intracellular lamellar body has dimensions of the order of 1 to 3 μm. The body contains a core which itself contains a small number of vesicles (*55*). Surrounding the core are lamellae which are "typical" trilaminar phospholipid bilayers. The periodicity of the lamellae has been variously reported to be 42 Å (*56,57*) and 66 Å (*54,58*). There is a thin outer matrix, in continuity with the core (*55*), and outside this matrix is the limiting membrane (*55*) which is lost when the lamellar body fuses with the plasma membrane during exocytosis (*22*). Once in the subphase, lamellar bodies apparently "unravel" to form tubular myelin.

Tubular Myelin

Tubular myelin was first described in the alveoli of lungs that had been exposed to a variety of toxic agents, e.g., silica (*59*), cortisone (*60*) and O_2 (*61*). It was later found that tubular myelin is a component of the normal EL (*62–64*), albeit in much smaller amounts than in lungs exposed to toxicants (*61,65*). In the normal lung tubular myelin is often found in alveolar niches (*29*). When sectioned transversely, tubular myelin appears as a quadratic lattice composed of lipid bilayers. (If cut longitudinally, it appears as a parallel array of lipid bilayers.) The distance between two bilayers is of the order of 400 Å (*29*) to 590 Å (*66,67*). Some tubular myelin is seen in inclusion bodies of alveolar macrophages but the physiological significance of this is unknown (*68*). Under the electron microscope, tubular myelin membranes can be seen in continuity with membranes of disrupted lamellar bodies (*22*). Also, lamellar bodies can be converted to tubular myelin *in vitro* (*67*). Freeze-etching studies indicate that lamellar body membranes are "smooth" (*69*), while membranes of tubular myelin contain particles at approximately the same periodicity as that of the tubular myelin lattice (*66,69,70*). Thus, it is considered that protein(s) have an important function in organizing the structure of tubular myelin. The function of tubular myelin is thought to be as a "reserve" form of surfactant, before the surfactant monolayer disperses onto the air–liquid interface.

Biochemical Composition and Toxicology of EL Components

The various classes of biomolecules of the EL are present in or on the hypophase; this is mainly aqueous in nature and the pH has been estimated to be approximately 6.9 *in vivo*, by direct measurement with a microelectrode (*71*). The hypophase also contains a store of reducing equivalent in the form of reduced ascorbic acid (*72*), which is present at a much higher concentration than that found in serum or lung tissue (*73*).

The most commonly used method of obtaining constituents of the EL for study is bronchoalveolar lavage, usually with a (buffered) saline or balanced salt solution. Although this method is a technically easy and "gentle" technique, it must be remembered that the whole of the lung-surface is lavaged. Thus, components of the EL may be contaminated with molecules derived from the upper airways, i.e., trachea and bronchi. Therefore, it is possible, particularly for compounds present in small amounts, that these compounds may be mistakenly assigned to the EL. Also lavage always contains free cells (usually macrophages in a normal animal), but these are easily removed by centrifugation.

Lipids

Both intracellular surfactant, i.e. lamellar bodies (*74–77*) and extracellular surfactant (*78–81*) have a similar chemical make-up in a variety of species (Table 1). Both are composed predominantly of lipid, usually with a small amount of protein, and trace amounts of carbohydrate (*78*). Over 80% of the lipid is phospholipid, the major one (60–80%) being phosphatidylcholine (*74, 78, 80*). A high proportion of the phosphatidylcholine is dipalmitoylphosphatidylcholine (DPPC), an unusual phospholipid in that it possesses two saturated fatty acids. DPPC is undoubtedly mainly responsible for the surface tension lowering abilities of surfactant (*82*). The second most abundant phospholipid is phosphatidylglycerol (*83,84*), which is normally present only in trace amounts in cells (*84*). Phosphatidylglycerol is also thought to be important in surfactant function by changing the physical properties of DPPC (*85*). The other phospholipids consist of small amounts of phosphatidylserine, phosphatidylinositol, phosphatidylethanolamine and lysophosphatidylcholine (*76,85,86*). The remainder of the total lipid is composed of cholesterol, cholesterol ester, triglycerides and free fatty acid (*74, 80,81*). It is not known whether the minor lipid components (excepting phosphatidylglycerol) play a

Table 1. Lipid composition of lamellar bodies and the extracellular lining.

	% of Total[a]								
	PC	PG	PS	PI	PE	Sph	Lyso PC	Others	Reference
Species									
Lamellar bodies									
Rat[b]	64.6	—		13.0[d]	6.4	—	—	7.7	(*45*)
Rat[b]	77.8	11.8	1.2	2.9	4.2	0.7	—	—	(*75*)
Rabbit[b]	86.0	5.0	0.4	4.2	3.5	0.5	0.7	—	(*76*)
Rabbit[b]	73.0	8.1	—	—	6.8	—	—	—	(*77*)
Extracellular lining									
Dog[c]	77.8	—	—	—	5.2	1.5	0.9	13.9	(*80*)
Sheep[c]	58.4	4.4	—	3.5	1.5	2.5	10.9	19.3	(*78*)
Ox[c]	70.6	1.8	—	2.8	3.1	1.3	0.2	20.2	(*78*)
Rat[c]	72.6	4.5	—	4.5	4.1	2.1	0.2	10.4	(*78*)
Rat[b]	82.1	7.8		4.1[d]	4.1	2.0	—	—	(*79*)
Rabbit[c]	83.4	2.5	—	1.6	1.1	2.0	—	9.0	

[a] PC = phosphatidylcholine; PG = phosphatidylglycerol; PS = phosphatidylserine; PI = phosphatidylinositol; PE = phosphatidylethanolamine; Sph = sphingomyelin; Lyso PC = lysophosphatidylcholine.
[b] % total phospholipid.
[c] % total lipid (includes free fatty acids, triglycerides, cholesterol, and cholesterol esters.
[d] PS + PI.

significant role in the function(s) of surfactant.

Changes in the amount of lipid in the EL as part of the response to toxicants have been reported several times. Increases in the amounts of lamellar bodies and/or tubular myelin in the alveoli, as revealed by histological observation have been described in lungs exposed to O_2 (*61*), silica (*59*), cortisone (*60*) and nickel dust (*87*). In extreme cases, after administration of drugs such as chlorphentermine (*88*), chlorcyclizine (*89*) or chloroquine (*89*), a phospholipidosis develops, which is characterized by the presence of lamellated inclusions in non-Type II cells of the lung (e.g., Type I, endothelial cells, fibroblast) as well as a buildup of lipid material in the airspaces. Quantitative studies, involving chemical assay of lipids in lung lavages or whole lung tissue, have also revealed large increases. For example, inhalation of chrysotile asbestos leads to an 11-fold increase in extracellular surfactant level (*90*) and intratracheal instillation of quartz results in a 12-fold increase in extracellular surfactant (*91*). Heppleston et al. (*92*) reported that total lung lipid increased 22 times after inhalation of quartz. Heppleston (*92*) termed this condition a lipo-proteinosis and under the light microscope the alveoli are filled with an amorphous PAS-positive material (*93*). A disease histologically similar to silica-induced lipo-proteinosis, called pulmonary alveolar proteinosis (PAP), is known in man (*94*). Its cause is unknown. Lung lavage fluid from patients with PAP contains large amounts of lipid and protein. Much of the lipid is in "particulate" form (*95*), consisting of disintegrating cells, cell debris, lamellar bodies and unusual vesicles and myelinlike structures (*95*). In lipo-proteinosis (*92*), PAP (*96*) and the increase in surfactant caused by chrysotile asbestos (*97*) there are no major changes in the composition of the lipid in the lavage or lung tissue.

The increase in levels of lipid is apparently due to enhanced synthesis versus normal degradation, as a result of exposure to asbestos (*97*) and silica (*98*). However, Ramirez-R and Harlan (*99*) postulated that synthesis of lipid was normal and degradation was decreased in PAP but this finding was questioned by Heppleston et al. (*98*). The increase in lipid is also probably related to Type II cell proliferation in the lung. Type II cells can divide to replace Type I cells that have been irreversibly damaged by toxic agents, e.g., NO_2 (*100*), apparently common lung response to epithelial injury. However, Type II cell hyperplasia to replace damaged Type I cells has only been definitively demonstrated after exposure to oxidant gases.

Decreases in lung lavage surfactant levels have been reported in animals exposed to radiation (*101*) or suffering from pneumonitis (*102*), and lower levels seem to be present in human smokers versus nonsmokers (*30,103*). Also, cadmium given to pregnant rats resulted in decreased phosphatidylcholine concentrations in the lungs of the fetuses (*104*). Reasons for the decrease were not apparent, but presumably Type II cells were damaged.

There are obvious dangers to the lung if surfactant levels are decreased, primarily, atelectasis may occur (*5,8*). In addition, increased surface tension on the alveolar surface leads to increased transudation of fluid and solutes (*105*) from the capillaries, which might result in pulmonary edema (*105*). Finally, the ability of the lung to defend itself against bacteria could be affected since surfactant is thought to help macrophages phagocytose and destroy bacteria (*106,107*). On the other hand, the significance of increased levels of surfactant to the lung are unknown. It is possible that the mechanical properties of the lung could be affected. In the case of the dust-induced increases, clearance of the deposited dust from the alveoli may be altered by

the amount of surfactant present. Also the chemical properties of the dust surface may be changed; surfactant can reduce the hemolytic activity of chrysotile asbestos *in vitro* (*108*), and this could affect the interaction of the dust with cells and hence its pathogenic effect. Much is still unknown about the effects of increased lipid levels on the lung.

In recent years, much attention has been paid to the possible role of metabolites of arachidonic acid (i.e., leukotrienes, thromboxanes and prostaglandins) in the development of some lung diseases, e.g., immediate hypersensitivity reactions. Many studies have shown that these compounds can be produced by cells present in the lung, e.g., macrophages, mast cells. However, there is little direct evidence, as yet, for the presence of these molecules in the extracellular lining, either in health or disease.

Glycolipids

Sahu et al. (*96*) reported that glycolipids, namely GM_3 ganglioside and GL_1 cerebroside, were present in rabbit lung lamellar bodies and particulate matter from lavage of patients with PAP. Slomiany et al. (*109,110*) described acidic (sulfated) and neutral glycosyldiacylglycerols in rabbit lung lavage, but these molecules may be contaminants from bronchial mucus. In all cases (*96,109,110*), glycolipids represented 3 to 4% (w/w) of the total lipid. Apparently nothing is known about the function(s) of these glycolipids in the EL or whether they are affected by toxic agents.

Glycosaminoglycans

The only glycosaminoglycan that has been detected in lung lavage is hyaluronic acid (HA), which comprises 2 to 3% of the total nonlipid material lavaged from human asthmatics (*111*). The source of the HA is thought to be the Type II pneumocyte (*112*). However, the lavage from healthy rabbits contains little or no HA (*113*). Increases in HA may be important in delayed hypersensitivity reactions of the lung, and possible other diseases, as HA has the ability to agglutinate alveolar macrophages (*113*).

Proteins

The question of whether specific surfactant apoproteins exist has caused much argument. Clements and King (*114*) reported that two apoproteins, approximate molecular weights 35,000 and 11,000, are present in surfactant from various species. Sawada and Kashiwamata (*115*) described an apoprotein (molecular weight 36,000) in bovine lung surfactant, and a phosphatidylcholine-binding protein has been found in rat lung lavage (*116*). Two unusual hydroxyproline-containing peptides (molecular weight 62,000 and 36,000) have been reported in lung lavage from people with PAP (*117*) and in normal lung lavage and lamellar bodies from rabbit (*118*). Lamellar bodies from pig lung, as well as pig surfactant, contain a highly hydrophobic group of proteins, molecular weights 11,000 to 16,000 (*119*). However, little detailed work has been done on the protein composition of lamellar bodies. On the other hand, various groups of investigators (*120–123*) have denied that surfactant apoproteins exist, and they postulate that proteins present in surfactant are an artifact of the isolation and preparation procedures. There is no apparent need for such apoproteins as the surface tension-lowering abilities of surfactant can be accomplished by pure lipid mixtures (*124*). It has been suggested by King and Macbeth (*125*) that the 34,000 mw apoprotein is necessary for fast adsorption of surfactant from the alveolar subphase onto the air–lipid interface but Metcalfe et al. (*126*) reported *in vitro* evidence against this supposition. Another possible function for the putative surfactant apoprotein is that of organizing the tubular myelin, for which protein is thought to be important (*69,70,127*).

Cell- and surfactant-free fractions of lung lavage contain a variety of proteins, most of which are probably derived from plasma (*28,30*) (Table 2). The concentration of plasma proteins in lavage effluents is similar to their concentration in serum with the most abundant being albumin, IgG, transferrin and a_1-antitrypsin. Albumin and IgG have also been histologically identified in the alveolar subphase of the EL (*29,128*). Presumably, the other proteins are also present in this location. The proteins are thought to be transferred from capillary to alveolus and vice-versa by means of pinocytosis through

Table 2. Plasma proteins in lavage effluents from the lungs of healthy humans[a]

Protein	% of Total (mean ± SEM)[b]
IgG	19.0 ± 1.7
IgA	10.2 ± 1.0
IgM	0.08 ± 0.04
IgD	0 ± 0
IgE	0 ± 0
β_1-Lipoprotein	0 ± 0
α_2-Macroglobulin	0.34 ± 0.18
Fibrinogen	0 ± 0
C_4	0.38 ± 0.03
C_3	0.98 ± 0.09
Ceruloplasmin	0.30 ± 0.03
Plasminogen	0 ± 0
C-reactive protein	0 ± 0
Haptoglobin	0.96 ± 0.20
Transferrin	5.6 ± 0.3
Hemopexin	0.85 ± 0.09
Albumin	52.5 ± 2.5
Prealbumin	0.09 ± 0.01
α_1-Antitrypsin	3.5 ± 0.3
Gc-globulin	0.24 ± 0.02
α_2-HS-glycoprotein	0.65 ± 0.06
α_1-Acid glycoprotein	0.74 ± 0.12
β_2-Glycoprotein 1	0.16 ± 0.02
Total	96.0

[a] From Bell et al. (*131*).
[b] n = 23.

the capillary endothelial cells and alveolar Type I cells (*29,128,129*). It is probable that some protein crosses the endothelium via intracellular clefts (*130*). There is a size restriction on the transport of proteins from plasma to alveolus—only those proteins with molecular weights of approximately 150,000 or lower have unrestricted transport (*28,131*). The only nonserum protein present in normal lung lavage, excepting "surfactant apoproteins," is secretory IgA—a specialized antibody which is specific to external secretions (*132*). Probably, most IgA in the lung is present in the upper airways (*133*), but small amounts may be associated with surfactant (*134*).

Presumably, IgG and IgA are present on the lung surface to protect the lung from viruses, bacteria, etc. As transferrin can have a bacteriostatic effect (*135, 136*), it also helps to protect the lung from bacterial attack, and α_1-antiprotease could prevent excess lung damage due to protease release from inflammatory cells (*137*). Whether the other serum proteins have specific functions in the EL is unknown.

An increase in lung lavage protein is often seen in the early phases of lung damage, e.g., as a result of exposure to O_2 (*138*), $CdCl_2$ (*139*), paraquat (*140*) and NO_2 (*141*). However, this increase probably represents intraalveolar edema—a relatively nonspecific indicator of lung damage. Indeed, Alpert et al. (*142*) found that passage of radiolabeled albumin from plasma to lung surface was a sensitive index of edema after inhalation of ozone (*142*). Longterm increases (weeks to months) in lung surface protein can be caused by radiation (*101*) or intratracheal instillation of quartz (*91*). Perhaps the most detailed studies have been performed on lavage from patients with PAP. This lavage contains large amounts of soluble protein in addition to great quantities of particulate material (*95*). In general, the protein composition of patient lavage is very similar to that of controls, but IgG and IgA concentrations were increased, and two apparently "new" proteins were found (*28,143*). Thus it seems that in PAP the increase in protein occurs without any loss of the selectivity and permeability of the blood-air barrier.

The particulate material from PAP lavage also contains large amounts of two glycoproteins, molecular weight 62,000 and 32,000 (*117, 144–148*), although these proteins are proteolytic fragments of a larger 80,000 molecular weight protein (*148*). The 80,000 protein is, in turn, a portion of a 250,000 molecular weight protein (*149*). This 250,000 molecular weight protein is probably synthesized and secreted by Type II pneumocytes (*150*). The parent protein, and its fragments, are unusual in that they possess short collagenlike sequences in their peptide chains (*144,146,148*). However, it is known that the proteins are not related to the complement component Clq.

A more subtle effect on lung surface protein has been detected in smokers. Although smokers have near normal amounts and composition of lavage protein (*131*), the α_1-antiprotease activity is reduced (*151,152*). The protein is probably inactivated by oxidants in cigarette smoke (*153,154*), which renders the protein functionally inactive, while still retaining antigenic reactivity in immunoassay techniques (*151,152*). This has obvious implications for the development of emphysema (*137*).

Fibronectin is a glycoprotein, found in tissue and plasma, which affects many interactions between cells and the extracellular matrix. Small amounts of fibronectin have been observed in lung lavage from normal humans (*155*). Increased levels of the protein were noted in lavage from patients suffering from interstitial lung disease (*156*), possibly as a result of increased synthesis by alveolar macrophages (*155*). The higher levels of fibronectin in interstitial disease may play a role in the pathogenesis of the syndrome, e.g., by affecting the functions of fibroblasts. Alternatively, fibronectin in the EL may be a "marker" for connective tissue turnover in the lung (*156*).

As with changes in lipid levels, the significance of increased levels of protein in the EL is not known. It is possible that the increase represents edema, but it does not seem likely that an animal could survive weeks or months with edema without becoming severely ill or dying. Perhaps the most likely explanation is that capillary permeability is increased as part of a chronic low level inflammatory process, thus leading to an increase in transport of protein from plasma to lung surface.

Enzymes

The only enzymes present in the normal EL of the rabbit are acid hydrolases such as α-mannosidase, β-hexosaminidase, acid phosphatase and an enzyme with an alkaline pH optimum, alkaline phosphatase (*31*) (Table 3). In addition Hayem et al. (*157*) reported the presence of β-hexosaminidase, α-mannosidase and β-galactosidase in lung lavage from normal humans. Nonspecific esterases have also been described in association with purified surfactant (*158*). The enzymes are found in the same compartment of the lung as the surfactant, i.e., the EL, and are probably derived from lamellar bodies (*31*) that appear to be modified lysosomes (*20,51–53*). The enzymes are thought to be released into the EL when the lamellar bodies are secreted onto the lung surface. There are differences between the enzyme compositions of lamellar bodies and lysosomes. Lamellar bodies contain very low amounts of some acid hydrolases, e.g., arylsulfatase, β-glucuronidase (*31*) and alkaline phosphatase, an enzyme not normally found in lysosomes. The alkaline phosphatase found in the EL is apparently lung specific (*159*). It is not known whether these various enzymes have any functions within the EL itself.

Most interest in changes in lung surfactant enzymes after exposure to toxic agents has been directed toward

Table 3. Hydrolases of the pulmonary extracellular lining of the rabbit.[a]

	Activity, μmole substrate hydrolyzed/30 min./total extracellular lining	% Total lung activity
α-Mannosidase	24.3 ± 4.6	14.6
β-*N*-Acetylglucosaminidase	69.0 ± 5.5	8.3
β-Galactosidase	2.7 ± 0.5	3.3
Acid phosphatase	6.6 ± 2.5	1.3
Alkaline phosphatase	11.3 ± 4.0	2.8

[a] From Hook (*31*).

the possibility of using differences in enzyme levels as markers of lung damage to aid in assessing the toxicity of various agents. A series of studies by Henderson and co-workers have investigated the effects of Triton X-100 (*160*), $CdCl_2$ (*139*) and NO_2 (*141*) on the lung. They measured enzyme activities such as acid phosphatase, alkaline phosphatase and lactate dehydrogenase, the last enzyme being present in very small amounts, if at all, in the normal EL (*31*). For all agents tested, there was a dose-dependent increase in the amounts of all the enzyme activities recovered by lavage (*139, 141, 160*). Also, there seemed to be a correlation between the extent of the increase and the extent of morphological damage in the lung tissue which was observed some time after the increase in the EL enzyme activities (*139, 141*). Similar results, measuring lactate dehydrogenase activity only, were reported by Roth (*140*) for bromobenzene, paraquat and other compounds and by Moores et al. (*161*) for intratracheal instillation of quartz. In the latter study (*161*), the increased levels of lactate dehydrogenase activity correlated well with the increased numbers of neutrophils on the lung surface, indicating that the cells were the major source of the enzyme, at least in this experiment. Thus, it seems that measurement of enzymes in the EL shows great promise as a short-term "screening" test for agents capable of damaging the lung.

Other workers have looked for the presence of enzymes, such as collagenase and elastase, in the lavage of patients suffering from various disorders, in the hope of gaining an insight into the pathogenesis of the disease as well as a possible means of diagnosing its severity. There is active collagenase in the lavage of people with idiopathic pulmonary fibrosis, although no elastase is present (*162*). High levels of elastase have been found in lavage from cases of pneumoconiosis (*163*) and patients suffering from adult respiratory distress syndrome (*164*). The enzymes are probably derived from activated alveolar macrophages (*165,166*) or neutrophils (*167*). The presence of the enzymes could indicate continuing connective tissue breakdown in the lung interstitium (*162*). If the restructuring process were to be altered in some way then this may lead to fibrosis of the connective tissue (*162*).

Immunology of the EL

The "immune system" of the EL is simpler than that of serum. Only two classes of antibody, A and G, are normally present (*28,30,131*). Most of the IgA is present as dimeric IgA (*133*) and is thought to be mainly present in the upper airways—the deep airspaces of the lung being protected by IgG (*133*). Some complement components, viz., C3, C4, and C6, have also been detected in lung lavage (*30,131*). The EL has higher concentrations of IgG and IgA compared to serum, probably as the result of local synthesis of antibody. Both IgA and IgG can by synthesized by a lung mince (*168*) and Kaltreider and Salmon (*169*) reported that B-lymphocyte plasma cells isolated by lavage could synthesize and secrete IgG. Thus the lung can produce antibody both in the interstitium and the airways (*133, 169*). T-lymphocytes are also present on the lung surface and can be obtained by lavage. These cells are less responsive than serum T-lymphocytes to certain stimuli. Part of the inhibition is due to the effect of surfactant on the cells (*170,171*), although the mechanism is unknown. Thus, the immune system of the lung may be functionally different from that of the rest of the body. The lung is known to be at least partly independent of the systemic immune system (*172*).

Elevated levels of IgG and/or IgA have been observed in the lung lavage from patients suffering a variety of diseases. IgG increases in idiopathic pulmonary fibrosis, chronic hypersensitivity pneumonitis and sarcoidosis (*173,174*). Both IgA and IgG are increased in pulmonary alveolar proteinosis and in pigeon breeder's disease (*28,175*). In at least one disease, sarcoidosis, the increased levels of antibody were correlated to increased numbers of IgG secretory cells in patient lung lavage. Also, the clinical severity of the disease seemed to be related to the number of IgG secreting cells. This provides evidence of the direct involvement of EL protein in this disease. However, whether this conclusion applies to other lung diseases, or whether the increased amounts of IgA and IgG in other syndromes is a result, rather than the cause of the disease, is unknown.

The EL in Pulmonary Toxicology

We have briefly reviewed and summarized our knowledge of the EL. The EL is a highly complex system with apparent functions which include and lie beyond its major accepted role as stabilizer of the alveoli and distal airways. Considering the critical importance of the EL to the lungs and mounting evidence, the EL must be identified as a target for toxic agents. Many questions have yet to be answered concerning the interactions between the EL and toxic agents: how do toxic agents produce alterations in the EL, how may the system change both in quality and/or quantity before pulmonary functions are seriously compromised, does a threshold exist beyond which pulmonary collapse is inevitable,

which aspects of the EL are particularly susceptible to toxic agents, and how and to what degree may the system protect itself? A great deal of research remains to be done before the role of the EL in pulmonary toxicology can be fully defined.

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